Disinfectant wipes are appropriate to control microbial bioburden from surfaces – the use of a new ASTM standard test protocol to demonstrate efficacy.

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Running Title: The role of wiping in environmental surface decontamination

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ABSTRACT

The use of disinfectant-pre-soaked wipes (DPW) to decontaminate high-touch environmental surfaces (HTES) by wiping is becoming increasingly widespread in the healthcare environment. However DPW are rarely tested using conditions simulating their field use and the label claims of environmental surface disinfectants seldom include wiping action. We report the use of the new ASTM International's standard E2967-15 specific to wipes by three independent laboratories, and the evaluation of five types of commercially available wipe products using this protocol particularly their ability to decontaminate surfaces and their ability to transfer acquired contamination to clean surfaces. All of the commercial DPW tested achieved a >4 $\log_{10}$ (> 99.99%) reduction in colony forming units (CFU) of *Staphylococcus aureus* and *Acinetobacter baumanii* with 10 seconds wiping, but only one DPW containing 0.5% accelerated H_2O_2 prevented the transfer of bacteria to another surface. This ASTM E2967-15 standard represents a significant advance in assessing DPW for microbial decontamination of HTES and should greatly assist R&D and in making more relevant and reliable claims on marketed DPW.

Introduction

High-touch environmental surfaces (HTES) are increasingly being recognised for their potential to spread pathogens in healthcare[1-5] and other settings.[6] Though HTES may on occasion introduce pathogens directly into patients, bare or gloved hands of healthcare workers are the more common vehicles after contact with such surfaces.[7] Appropriate decontamination of HTES can reduce the risk of contamination of the hands of healthcare personnel.[8] Decontamination of HTES is almost always either by a disinfectant-spray-and-wipe procedure or by wiping with a disinfectant pre-soaked wipe (DPW), in both cases combining the microbicidal action of the disinfectant with physical removal by wiping.[9] However, products marketed for this purpose are rarely tested using conditions simulating their field use and label claims of environmental surface disinfectants seldom include the wiping action. Short-comings in the available methods for testing the decontaminating potential of wipes have been identified and can be divided broadly into i) wiping action not controlled (e.g. AOAC International 961.02; EN16615 “4-Field test”, ASTM International E2362, US EPA Method for Disinfection Using Pre-Saturated Towelettes) or/and ii) inappropriate contact time (e.g. AOAC International 961.02, US EPA Method for Disinfection Using Pre-Saturated Towelettes).[9] The lack of an appropriate test protocol for measuring the activity of antimicrobial wipes has been recognised by the Royal College of Nursing,[10] which suggested the use of surface efficacy tests in the absence of a better test, but also recognised the use of non-standard tests under specific conditions, notably a contact time of less than 30 seconds.[10] The 3-stage test purposefully designed to measure the activity of antimicrobial wipes, in combination with the purpose build Wiperator (http://www.filtaflex.ca/wiperator.htm; accessed 20/04/15), has recently been recognised as an ASTM international standard.[11] The 3-stage test has been used to measure the activity of disinfectant-,[12-14] sporicide-[15] and detergent-containing wipes.[16] This study reports on the efficacy of five types of commercially-available DPW against two types of common vegetative bacterial pathogens using the new ASTM E2967-15 international standard[11] by

three independent laboratories.

Materials and Methods

Test bacteria

Staphylococcus aureus (ATCC 6538) and *Acinetobacter baumannii* (ATCC 19568) were chosen as Gram-positive and Gram-negative species, respectively, as archetypal healthcare associated bacterial pathogens. The two species are also relatively resistant to drying, thus allowing viability concentration of $\geq 10^7$ CFU on each dried carrier.

Both the organisms were grown at 36 ± 1°C for 18 ± 2 h in 10 mL tryptic soy broth (TSB). The suspension was then centrifuged at 3,000 *g* for 20 min, and the pellet resuspend in 5 mL of tryptone-sodium chloride (TSC; 1 g Tryptone and 8.5 g of NaCl in 1 L of double-distilled water). Tryptic soy agar (TSA) was used to recover viable bacteria from control and test samples.

Soil load

A 3% (w/v) stock solution was prepared by adding 3.0 g of bovine serum albumin (BSA) to 100 mL of TSC. It was passed through a 33 mm diameter membrane filter (Millipore) with a nominal pore diameter of 0.22 µm, aliquoted in 5 mL-volumes and stored at 4 ± 2°C. One part of the soil load was added to nine parts of the test bacterial suspension to give a final concentration of 0.3% w/v BSA.

Carriers

Disks (AISI Type 430; 1 cm in diameter and 0.7 mm thick) of magnetized and brushed stainless steel were used as prototypical HTES.[17,18] Before use, each disk was inspected under a dissecting microscope (20-fold magnification) to ensure freedom from any defects and/or rusting. Up to 20 clean disks were placed in a Nalgene vial and the vial loosely capped before autoclaving. Each disk was used only once and then discarded.

Wipes

Three manufacturers provided samples of their respective wipes along with the Materials Safety Data Sheets. The samples were labelled as A-F and stored at room temperature. The main types of ingredients contained in the wipes are given in Table 1. The wipe formulations are proprietary information and to maintain product anonymity no other information is provided. Wipes A and B were different batches of the same wipe; wipes C to F were different makes. A and B were included to aid in assessing the reproducibility of the method. All commercial wipes were tested within the shelf life indicated on their respective labels. Unmedicated J-Cloth towels, composed of cellulosic fibres from wood pulp, were purchased locally by the participating labs and used as controls. While disinfectant-free pieces of the tested DPW could also be pre-soaked with a buffer and used as controls, it is often difficult to obtain such 'blanks' from manufacturers. Besides, they are often highly hydrophobic and cannot be readily wetted without the addition of detergents and prolonged soaking with potential impacts on the quality of the test data. J-Cloth was selected as the control wipe because it is readily available, absorbent, lacks any inherent microbicidal potential and could be autoclave sterilized.

Neutraliser

The neutraliser consisted of 30 g of Saponin, 1 g L-histidine, 30 g polysorbate-80, 3 g azolectin from soybean, and 5 g of sodium thiosulfate in 1 L of TSC.

Test procedure

The ASTM E2967-15 protocol[11] was followed. Briefly:

Inoculation of carriers

Working in an operating biosafety cabinet, 20 sterilized disks were placed in a 100 mm diameter sterile plastic Petri dish. A calibrated positive-displacement pipette (Eppendorf) was

used to place 10 µL of the test bacterial suspension with BSA (0.3% w/v) at the centre of each disk, but without spreading the inoculum. For consistency, the same pipette tip was used throughout to inoculate a given batch of disks. The Petri dish with the inoculated disks was transferred to a 36 ± 1°C incubator for 30 minutes to dry the bacterial suspension.

Preparation of wipes for testing

Before removing a wipe for testing, its container was inverted once for 5 s and again for 10 s to uniformly wet its contents. Wearing sterile gloves, the first three wipes were removed and discarded, and a 4 cm x 4 cm piece cut out from the fourth one using a sterile pair of scissors. The piece was mounted as a sterile single layer on the boss provided with the Wiperator. The wipe cutting- and mounting-steps were carried out aseptically as quickly as possible to avoid extraneous microbial contamination and also to minimize the evaporation of the disinfectant liquid in the wipe. For the control, a 4 x 4 cm piece of autoclave-sterilized J-Cloth wetted with 320 µL TSC was processed in the same way as the test wipes.

Wiping, recovery of bacteria dried on disk surface and transfer to a clean disk

A piece of test or control wipe was mounted on the Teflon boss with an O-ring of the Wiperator. The disks were wiped using an orbital motion for 10 seconds at a pressure of 150 g. The wiped disk was removed using sterile forceps and transfer to a vial containing 1 g of sterile borosilicate glass beads and 1 mL of neutralizer. After 30 sec of vortexing, the test suspension was serially diluted in TSC and appropriate 10-fold dilutions placed onto TSA plates using the Miles & Misra method.[19] The inoculated plates were held upright at room temperature for 30 ± 2 min and then incubated at 36 ± 1°C for 48 ± 2 h.

To measure transfer, following the first wiping and without removing the mounted wipe on the Teflon boss a sterile stainless steel disk was wiped for 10 seconds at a pressure of 150 g. The disk was then removed and processed as described above.

Controls

The efficiency of recovery of the test bacteria from the disks was assessed by placing 10 µL (≈10^7 CFU) of the test inoculum onto a disk and eluting it immediately. The number of CFU recovered from the disk was compared to the number of CFU placed on it in the first place. The number of CFU surviving the drying process on the disks was measured by depositing 10 µL (≈10^7 CFU) of the test suspension onto a disk followed by drying as described above. Once the inocula were dry, disks were eluted and assayed for CFU. The average CFU counts from three eluates were used as the 'baseline' for calculating the $\log_{10}$ values in CFU after wiping and transfer.

To measure the efficacy of the neutralising process, a sterile disk was wiped with a test wipe, placed in 1 mL of neutralizer and then immediately followed by the addition of 10 µL (200-400 CFU) of the test bacterial inoculum. After a 15-min contact time at 22 ± 2°C, the eluates were plated on TSA plates and incubated as described above. Another disk was wiped with J-Cloth wetted with 320 µL TSC, eluted and processed as described above. Effective neutralization of any microbicidal activity on the disks was considered to have occurred if the number of CFU from the test eluates was ≥ 0.5 times the control. This figure is adapted from EN standards such as EN 1276,[20] which has similar requirements. Randomly selected disks were tested for sterility, and sterility tests were also performed on all media and reagents as a part of our routine quality control.

Participating laboratories

Three laboratories performed the ASTM E2967-15 using the same protocol, wipe material (same lot number) and bacteria (same standard strain number). Visits between laboratories and regular teleconferencing were set up to ensure that the same procedure was adhered to. A single operator in each laboratory performed the test for the duration of the study to increase repeatability. The laboratories were (1) the School of Pharmacy & Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, (2) Hospital Infection Research Laboratory, Queen Elizabeth Hospital Birmingham, Birmingham, England, and (3) Faculty of Medicine, Univ. of Ottawa, Ottawa, ON, Canada.

Analysis of results

All experiments were performed in triplicate. The data were analysed as described in the ASTM standard E691-12.[21] The precision of the test method was determined by evaluating the repeatability and reproducibility of the test protocol.[21] A one-way analysis of variance (within- and between-laboratories) was used separately for each type of wipe to estimate the precision statistics from the collected data.

RESULTS

The ASTM E2967-15 standard includes drying the bacterial inocula on stainless steel surface followed by their recovery from that surface. It has been well established that the drying process may be detrimental to microbial viability. Here from an initial inoculum of 7.42 $\log_{10}$ for *S. aureus* and 7.25 $\log_{10}$ *A. baumannii* (average value for the 3 laboratories; data not shown), the mean loss in viability for *S. aureus* and *A. baumannii* from the drying process used here was 0.23 and 0.52 $\log_{10}$, respectively (average value for the 3 laboratories). Such a high efficiency of recovery showed that there was virtually no irreversible attachment of the bacteria to the carrier surface and also that the elution procedure and the neutralizer had no effect on the viability of both the test organisms.
As is required in efficacy tests,[17] neutralisation of the microbicidal activity of the tested DPW immediately at the end of the contact time was determined. Though a neutralizer with the same composition was used throughout the study, its activity was first validated against each type of DPW and the bacterial species tested. All of the wipes tested were successfully neutralized as assessed by the criteria in EN standards (Table 2), It should also be noted here that values beyond 100% are not unusual because detergents added to microbicides may disaggregate bacterial clumps resulting in increased numbers of CFU in test suspensions.

The testing for bacterial removal and transfer was with a wiping time of 10 sec. This time was selected based on observations on the use of DPW by healthcare staff in an intensive-care unit.[14] All of the wipes tested produced a significant ($p < 0.001$, Mann – Whitney U test) reduction in bacterial concentration on surfaces compared to the control wipe. Wipes A, B and E were shown to produce a 7.0 $\log_{10}$ CFU reduction or above of both test bacteria from the surface following wiping (Fig. 1). Wipes C, D and F produced a better reduction of the Gram-negative than the Gram-positive on surface overall. Wipe C was the least effective but still produced a >5 $\log_{10}$ reduction in *A. baumannii* and >4 $\log_{10}$ reduction in *S. aureus* (Fig. 1).

During routine wiping, DPW may dislodge localized contamination on HTES and transfer it to neighbouring areas being wiped posing a substantial threat to the spread of pathogenic bacteria.[14] Table 3 shows the total number of carriers used in the study (all laboratories combined), the number of carriers with recovered CFU following wiping, and the number of carriers positive for bacteria following transfer. Wipes A and B showed no detectable transfers of CFU of either test bacteria following wiping. However, there was recovery and transfer of *S. aureus* from wipes C, D and F (amounting to the following means ± standard deviations: 2.13 ± 0.59, 0.62 ± 0.80 and 1.09 ± 1.39 $\log_{10}$ CFU transferred, respectively). No recovery or transfer of *S. aureus* from wipe E was recorded. Although there was recovery of viable *A. baumannii* from wipes C-F, only wipe F transferred the Gram-negative bacteria (0.43 ± 0.89 $\log_{10}$ CFU transferred). The control wipe transferred both bacteria for all of the surfaces tested (3.86 ± 0.77 and 3.92 ± 0.47 $\log_{10}$ CFU transferred for *S. aureus* and *A. baumannii,* respectively).

The precision of the test method was determined by evaluating the repeatability and reproducibility of the test protocol. The critical values for the consistency statistics at the 0.5% significance level were obtained from the ASTM standard E691-12[21] and were 1.15 for the "*h*-value" (which measures the consistency of the test results between laboratories) and 1.67 for the "*k*-value" (which measures within-laboratory precision). Concerning the "*h*-value", all laboratories have positive and negative values for the tested wipes and no values

exceeded the critical value. There were also no extreme values recorded. With this in mind, the consistency of the test results from laboratory to laboratory was acceptable according to the ASTM standard E691-12[21] (Fig.2). Concerning the "*k*-value" and *A. baumannii*, two laboratories presented high "*k*-values", which were above the critical value (Fig. 3). Further analysis of the data, notably the plot of the "*k*-values" per laboratory revealed that the plot for wipe F for laboratory-2 for *S. aureus* was different from the other 2 laboratories, likewise for *A. baumannii* with wipe D for laboratory-3 and wipe E for laboratory-2 (Fig. 3). With these unusual plot patterns identified, procedure and data were checked and no explanation could be found for such a deviation in values. Thus, in accordance to the ASTM standard E691-12[21] all data were used to calculate the 95% repeatability '*r*' (the value below which the absolute difference between two individual test results obtained in the same laboratory may be expected to occur with $p \approx 0.95$) and reproducibility '*R*' (the value below which the absolute difference between two test results obtained in different laboratories may be expected to occur with $p \approx 0.95$) limits. The repeatability and reproducibility limits were acceptable regardless of the bacteria tested (Table 4).

DISCUSSION

DPW are increasingly being used in healthcare settings. They provide the advantage of combining the mechanical action of microbial removal with the killing action of the disinfectant.[1,5,9,10,12,13,14,15,16] Microbial decontamination of environmental surfaces by wiping is subject to many variables, and failure to standardize them properly during testing of wipes may give inconsistent test data.[9] In particular, precise control of the pressure applied during wiping, the normally brief wiping times of a few seconds as well as the style and number of wiping strokes are all difficult to standardise without a programmable mechanical device. Initial testing with volunteers using a single-pan digital-readout balance gave a range of 100-300 g of pressure during wiping.[22] Until the publication of the ASTM E2967-15 standard[11], efficacy tests did not simulate conditions of use whereby the mechanical action on the

weight exerted on the wipes are taken into account.[9] Furthermore, following observation of wipe usage in practice, it became apparent that one wipe can be used on multiple surfaces,[14] thereby increasing the risk of releasing microorganisms picked up on surfaces.[13,14,15,16]

The two types of bacteria in this study were selected based on their importance as healthcare-associated pathogens known to spread via HTES, ability to withstand inoculum drying much better than commonly used test organisms such as *Escherichia coli* and *Klebsiella pneumoniae*, and ease of culture and quantitation yielding relatively high CFU in 48 h or less.

This multi-laboratory study was designed to measure the variability in results due to factors inherent in the new ASTM E2967-15 standard[11]. To that end, data were analysed to calculate the repeatability and reproducibility of the removal-by-wiping part of the method for each bacterium. The results demonstrated that the repeatability (*r*) and reproducibility limits (*R*) did not remain constant between the different wipes used (Table 4). This was to be expected as wipes tested had different materials and formulations (to note the exact composition of the wipes is proprietary data and are not available). Overall, all reproducibility limits were higher than the repeatability ones. Some reproducibility limit values were large for some material/bacteria combinations (Table 4). Since no issue with the experimental procedure or operator was identified, such data possibly suggest some variability in the material tested despite the same batches being tested in all three laboratories. We recently highlighted that the formulation used in wipes and notably the ratio between surfactant/disinfectant and the type of materials play an important role in the ability of the wipe to remove and/or kill bacteria on surfaces (Sattar and Maillard 2013).[9] The precision statistics ought not be treated as exact mathematical quantities, applicable to all circumstances and uses. The repeatability and reproducibility limits may be considered as general guides, the 95% probabilities serving as rough indicators of what can be expected (ASTM E691-12) [21]. Be that as it may, we would propose that the repeatability and reproducibility limits calculated for the wipes suggest that the combination of the Wiperator

and the 3-stage test as described in ASTM E2967-15[11] is appropriate to test the efficacy of antimicrobial wipes for the use in healthcare and other environments. Indeed the purpose-built Wiperator has been designed and tested with these crucial factors in mind. It simulates the orbital action of wiping and other parameters based on observations in healthcare settings (William et al., 2009).[14] It permits pre-setting of pressure during contact, duration of wiping as well as the number of wiping strokes in a given test, thus allowing for greater precision and reproducibility.

As shown here, the correct use of properly formulated DPW can be quite effective in ridding HTES of $>10^4$ CFU of potentially harmful bacteria with a 10-sec wipe with minimal risk of transfer of viable organisms to neighbouring surfaces from the used DPW. In view of this, it is strongly recommended that the current practice of label claims on antimicrobial wipe packaging with field-irrelevant contact times be discouraged and replaced with use directions based on their assessment with wiping action included. Further work with DPW containing other levels/classes of actives as well as other types of HTES-borne pathogens will be needed to set practical and realistic product performance criteria acceptable to the industry and the regulators alike. Such a move will also provide end-users with better confidence in label claims while potentially contributing to better infection control and prevention.

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Conflict of interest

GAMA Healthcare, Nice-Pak International Limited and Virox provided the financial support to the three institutions to perform the study.

Dr S Sattar is a member of the Board of Directors of Virox Technologies.

Dr T Sharpe is the manufacturer of the Wiperator. At the moment, the Wiperator is available from a single source. However, it has not been patented and is available for purchase or license, or details for its construction are available upon request.

All the other authors: no conflict of interest to declare.

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Table 1. Main type of ingredients contained in the tested wipes.*

Wipe code	Active ingredient(s)
A/B	Oxidising agent
C	Mixture of cationic biocides
D	Peroxygen releasing formulation
E	Chlorine releasing agent
F	Mixture of alcohols and quaternary ammonium compounds

* The wipe formulations are proprietary information and to maintain product anonymity no other information is provided

Table 2 Wipe neutralization based on % recovery of CFU. Consolidated data from the three laboratories.

	% Recovery of CFU	
Wipe	***S. aureus***	***A. baumannii***
A	73.73	84.77
B	126.79	145.25
C	99.43	158.25
D	67.42	95.41
E	101.10	105.78
F	96.37	128.85

Table 3 Total number of carriers tested for the 10 sec. experiments and number of carriers with CFU recovery used to calculate mean $\log_{10}$ reductions of test and transfers (data collated from all three laboratories)

Test microorganism	**Test**		**Total number of carriers/number positive***					
		Control	**A**	**B**	**C**	**D**	**E**	**F**
Staphylococcus aureus	Removal	15/15	15/0	15/0	15/15	15/13	15/0	15/12
	Transfer	15/15	15/0	15/0	15/15	15/6	15/0	15/6
Acinetobacter baumannii	Removal	11/11	15/0	15/0	15/12	15/9	15/11	15/13
	Transfer	11/11	15/0	15/0	15/0	15/0	15/0	15/3

* a ‘0’ indicates there was no viable bacteria left on the surface of the disk

Table 4 Precision statistics for the efficacy of wipes to remove bacteria from surfaces.

Wipes	$\bar{x}$	$S_{\bar{x}}$	S_r	S_R	r	R
Staphylococcus aureus						
A	7.4767	0.4922	0.0509	0.4939	0.14	1.38
B	7.4022	0.6216	0.1327	0.6310	0.37	1.77
C	4.3883	0.9922	0.3863	1.0412	1.08	2.92
D	6.2767	0.7163	0.8670	1.0071	2.43	2.82
E	7.4767	0.4922	0.0509	0.4939	0.14	1.38
F	6.1028	1.4791	0.5700	1.5506	1.60	4.34
Acinetobacter baumanii						
A	7.3556	0.2656	0.0623	0.2704	0.17	0.76
B	7.1344	0.5205	0.0878	0.5254	0.25	1.47
C	5.7389	0.5782	1.1006	1.1006	3.08	3.08
D	6.9072	0.8721	0.5518	0.9816	1.54	2.75
E	6.7367	1.3158	0.9387	1.5227	2.63	4.26
F	5.8678	0.2957	1.0580	1.0580	2.96	2.96

$\bar{x}$: mean (cell average)
$S_{\bar{x}}$: mean (cell average) standard deviation
S_r: repeatability standard deviation
S_R: reproducibility standard deviation
R: reproducibility limit
r: repeatability limit

Fig. 1 Log_{10} reductions in CFU with 10 sec wiping: Consolidated data from all three laboratories. (white): *Staphylococcus aureus*; (grey) *Acinetobacter baumannii*

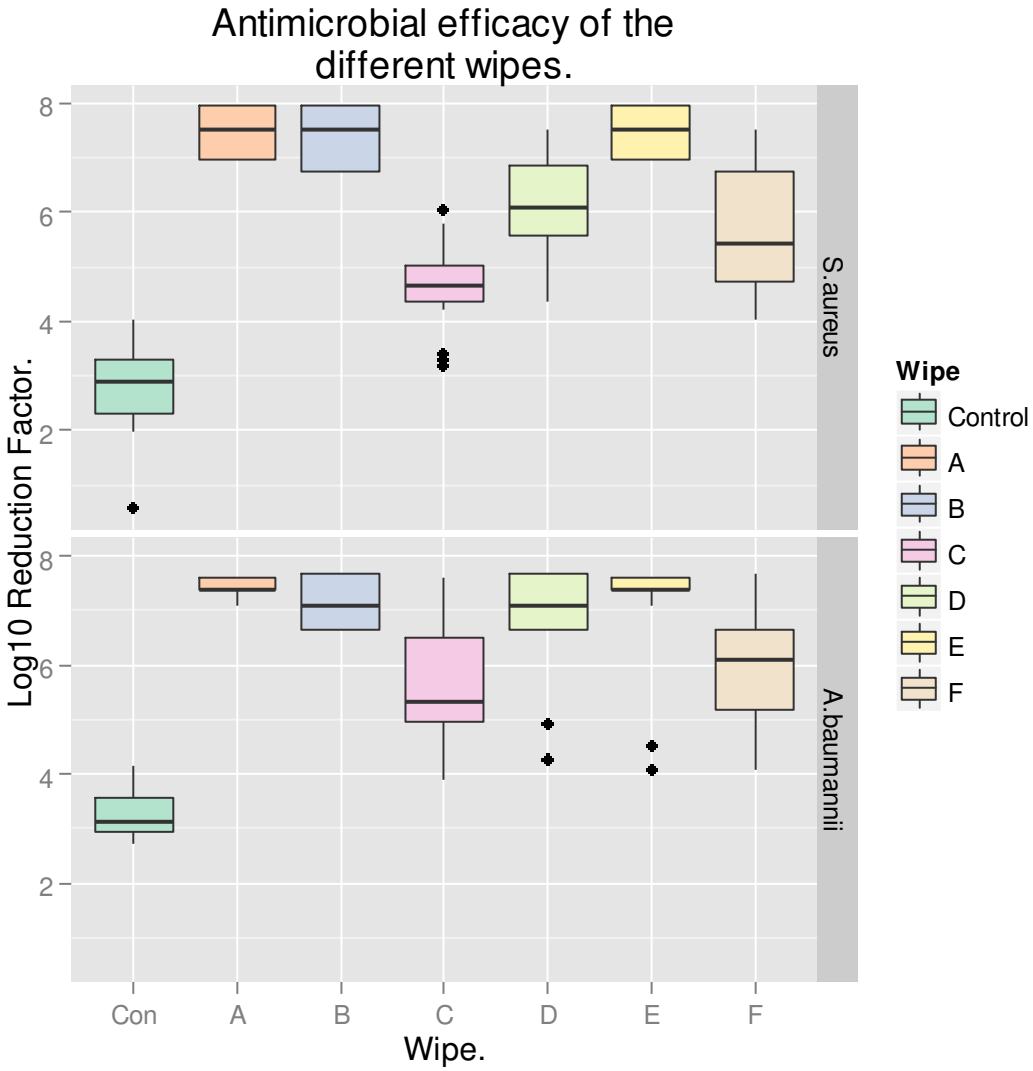

Fig. 2 **"***h*-values" obtained from the removal of a) *S. aureus* and b) *A. baumanii* from surfaces following mechanical wiping. Wipes are labelled A-F, and laboratories are denominated 1 to 3. The critical values of the *h* consistency statistic at the 0.5% significance level is 1.15 (see text). Doted lines correspond to the critical value.

a) b)

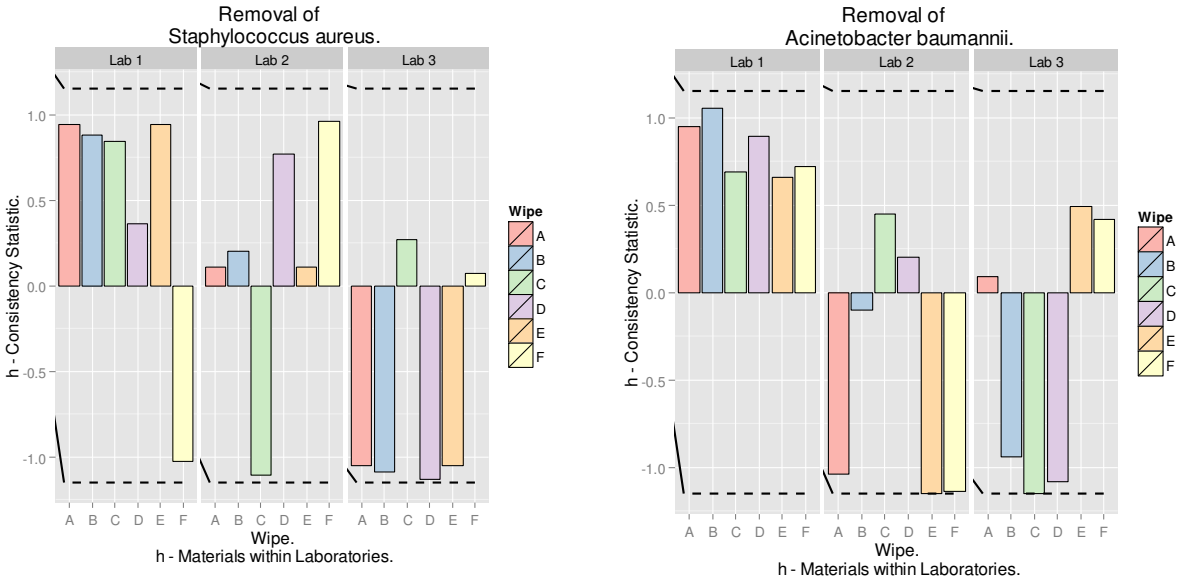

Fig. 3 **"***k*-values" obtained from the removal of a) *S. aureus* and b) *A. baumanii* from surfaces following mechanical wiping. Wipes are labelled A-C, and laboratories are denominated 1 to 3. The critical values of the *k* consistency statistic at the 0.5% significance level is 1.67 (see text). Doted lines correspond to the critical value.

a) b)